Poster 033 Follicle homing antigen presenting cells modulate TH2 bias

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Conditional knockout of CXCR5 on CD11c+ cells prevents protective T_H2 response following *T. muris* infection

Introduction

The expression of the chemokine receptor CXCR5 by dendritic cells and their homing to B-cell follicles are suggested requirements for the generation of T-helper type 2 (TH2) cells in response to infection. Previous studies revealed that bone marrow chimeric mice deficient in CXCR5 in dendritic cells or CD4+ T-cells impaired the development of both T-follicular helper (T_{FH}) or T_H2 cells after infection[1]. *Trichuris muris (T. muris)* is a gastrointestinal parasite capable of naturally infecting mice, and is used as a model for the human parasite *Trichuris trichuria* which affects over 1 billion people in predominantly developing countries. Infection with *Trichuris spp*. elicit a spectral immune response. A strong T_H2 response results in immunity and expulsion of the parasites, whereas a T_H1-biased response results in susceptibility and persistent infection. High dose infection with *T. muris* stimulates a T_H2-dominated response in resistant mouse strains such as C57Bl/6 with worm clearance within 21 days. We therefore infected CD11cCre:CXCR5fl and CXCR5fl (control) transgenic mice with *T. muris* and monitored their response to infection

Methods

LoxP **sites were gene-targeted flanking the open reading frame (ORF) of the CXCR5 gene in C57Bl/6 mice. CXCR5fl mice were bred to homozygosity with or without CD11cCre[2]. Mice were gavaged with ~250 embryonated *T. muris* eggs and sacrificed 30 days post infection.**

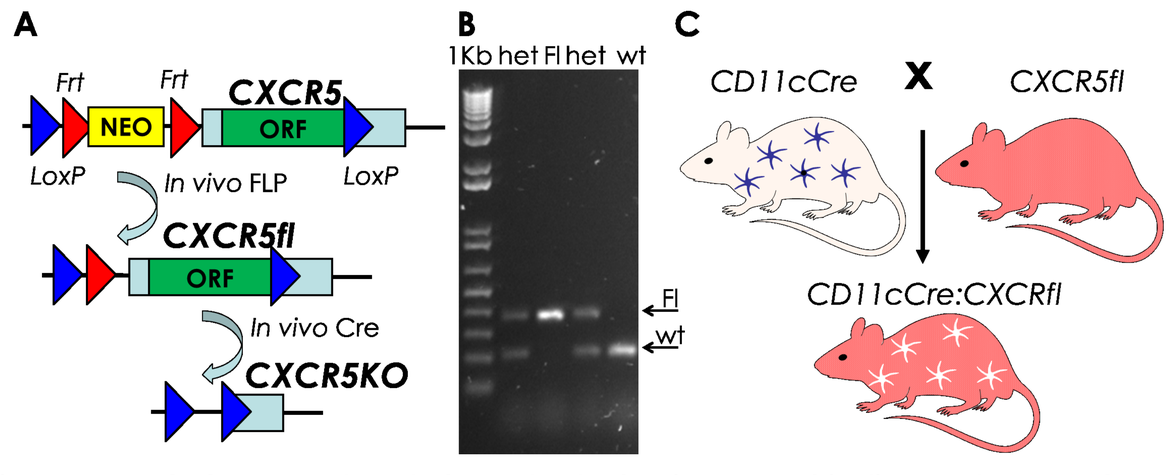

Figure 1. A. CXCR5 gene-targeted construct, showing recombination post FLP or Cre. B. Genotyping of CXCR5fl allele via primers specific to the regions surrounding the 5' *LoxP* site. C. Generation of CD11c-restricted CXCR5 knockout, CXCR5fl alleles were bred to homozygosity whilst incorporating the CD11cCre transgene. Resultant offspring express possess a CD11c-specific knockout of CXCR5 (CD11cCre:CXCR5fl).

Results

CD11cCre:CXCR5fl transgenic mice are persistently infected with *T. muris* 30 days post infection (d.p.i.) unlike CXCR5fl (control) mice

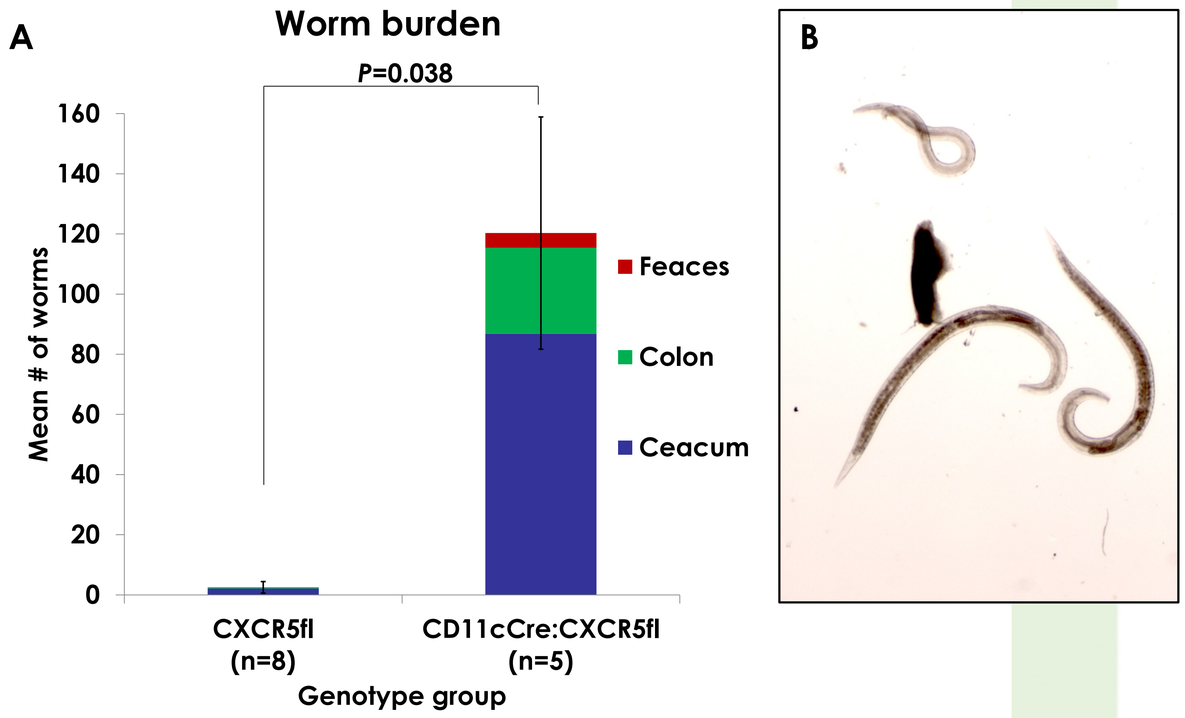

Figure 2 A. Adult worm burden at 30 d.p.i. Whole adult worms were counted in the ceacum, colon and faecal contents 30 days after infection with ~250 embryonated *T. muris* eggs. CXCR5Fl mice revealed mostly clearance of parasite (range 0-16 adult worms), CD11cCre:CXCR5fl mice were all persistently infected (range 71-272 adult worms) B. Adult *T. muris* isolated from CD11cCre:CXCR5fl ceacum at 30 d.p.i.

Changes to cytokine profile induced by *T.muris* infection in CD11cCre:CXCR5fl mice

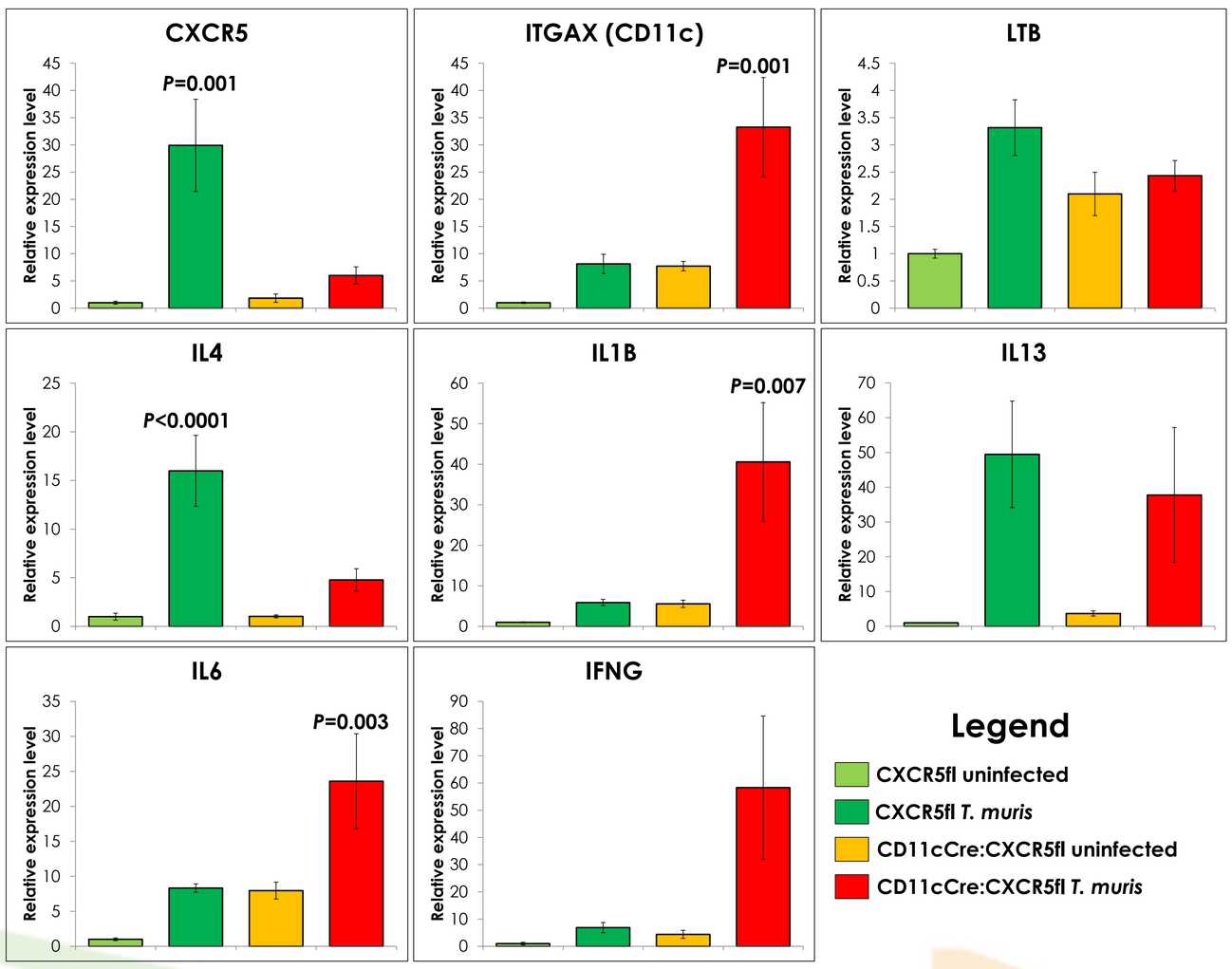

Figure 3 Gene expression profiling of mesenteric lymph nodes at 30 d.p.i. Following mRNA extraction and cDNA synthesis, primer-specific QPCR were performed for various cytokine and immune marker genes. Gene expression was normalised to control gene RPL19 and expressed as relative fold change in expression level when compared to CXCR5fl uninfected mice.

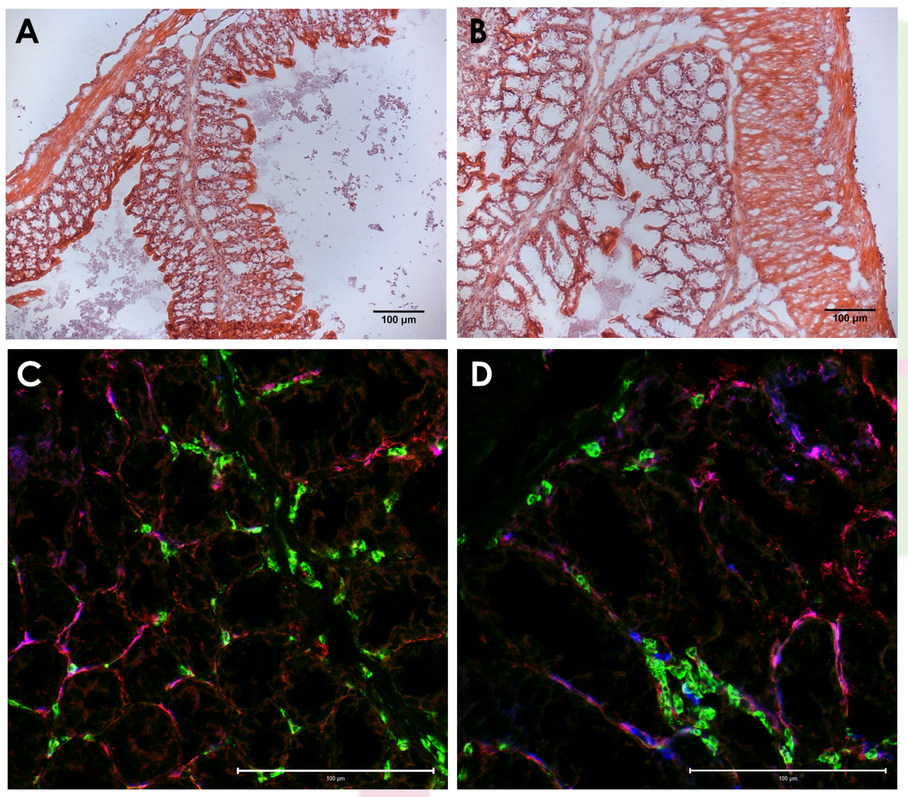

Figure 4 (Left) H&E stained sections of colon at 30 d.p.i. from A, CXCR5fl and B, CD11cCre:CXCR5fl mice reveal inflammation in large intestine concurrent with persistent *T. muris* infection. Scale bars = 100 µm

Investigation of myeloid cell distribution in large intestine reveals a uniform distribution of CD11b+ cells in CXCR5fl mice (C), unlike focal accumulation of CD11b+ cells in CD11cCre:CXCR5fl mice (D). Scale bars = 100 µm

CD11b
CD11c
B220

Conclusions

The expression of the chemokine receptor CXCR5 by dendritic cells and their homing to B-cell follicles are suggested requirements for the generation of T-helper type 2 (TH2) cells in response to infection. Conditional knockout of the chemokine receptor CXCR5 in CD11c+ cells renders C57Bl/6 mice susceptible to high dose *T. muris* infection. Cytokine profiling from mesenteric lymph nodes revealed an increase in CD11c+ cells in the absence of increased CXCR5 expression and altered cytokine profile with reduced IL4 and increased IL1B, IL6 and IFNG. Persistently infected CD11cCre:CXCR5fl mice reveal inflammatory changes within the colon with recruitment of CD11b+ cells to specific areas.

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References: 1. Leon, B. et al. 2012 Nat Immunol 13(7), 2. Caton, M. L., et al 2007 J Exp Med 204(7)

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